I. Status of the Claims

Claims 1-7 are pending in the application and stand rejected. Claims 1-3 and 5 have been amended, and claim 8 has been canceled. No new matter has been added.

II. Amendments

The claims have been amended to more clearly define the invention. Specifically, claims 1, 2, and 3 have been amended to replace the term "bioactive agent" with the term "nucleic acid." Support for this amendment can be found in claim 3 as-filed and in the specification at page 23, lines 22 to 24, which explains that the bioactive agent can be a nucleic acid. Claim 3 recites that the nucleic acid is DNA or RNA. The specification states that that the nucleic acids can be DNA or RNA. *Specification* at p. 23, ll. 22-24. The claims have also been amended for clarity by replacing the phrase "complexing agent" with "polycation."

Claims 1 and 2 have been amended to recite a "water-in-oil emulsion," and to recite that the water-in-oil emulsion comprises "lipid stabilized water droplets." These amendments are supported by the specification, for example, at page 27, lines 8-12. This amendment more clearly defines the invention by providing antecedent basis for the phrase "lipid stabilized water droplet" used later in the claim. Support for this amendment can also be found, for example, on page 11, lines 1-6, which describes phospholipid stabilized water droplets depicted in Figure 2.

Claims 1 and 2 also have been amended to more clearly define the invention by adding the phrase "wherein the nucleic acid to lipid ratio is at least 0.5 μg nucleic acid per μmole of liposomal lipid." Support for this amendment can be found, for example, in the specification at page 23, lines 24 to 28. Claims 1 and 2 have been further amended for clarity by replacing the word "suspension" in

step (e) with the word "emulsion." Support for this amendment can be found at page 33, lines 1-18, which describes an example of the present invention.

III. Remarks

A. Claim Rejections Under 35 U.S.C. § 103(a)

1. Rejection over Szoka in view of Gao or Papahadjopoulos, and optionally further in view of Tikchonenko

Claims 1-7 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over *Proc. Natl. Acad. Sci. USA*, 1978, 75, 4194-4198 by Szoka et al. ("Szoka") in view of either U.S. Patent No. 5,795,587 to Gao et al. ("Gao") or in view of U.S. Patent No. 6,071,533 to Papahadjopoulos et al. ("Papahadjopoulos"), optionally further in combination with *Gene*, 1988, 63, 321-330 by Tikchonenko ("Tikchonenko"). Applicants respectfully traverse.

In order to establish a *prima facie* case of obviousness, the Examiner must determine the scope and content of the prior art, ascertain the differences between the claimed invention and the prior art and resolve the level of ordinary skill in the pertinent art. *Graham v. John Deere Co.*, 383 U.S. 1, 148 (1966). Once the Graham factual inquiries have been resolved, the Examiner must explain why the differences between the cited references and the claims would have been obvious to one of ordinary skill in the art. Fed. Reg. Vol. 72, No. 195, p. 57527. The Supreme Court in *KSR* stressed that "obviousness cannot be sustained by mere conclusory statements; instead there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR* 127 S.Ct. 1727, 1740 (2007); see also Fed. Reg. Vol. 72, No. 195, p. 57529. "The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. Fed. Reg. Vol. 72, No. 195 at p. 57528.

Applicants submit that the cited reference combination fails to teach or suggest each and every limitation of the present claims, and furthermore, the Examiner has not provided any articulated reasoning or rational underpinning for combining the cited references in order to arrive at Applicants' claimed invention.

The present invention not only recites the use of a complexing agent to condense nucleic acids, but the claims recite the addition of the complexing agent under particular conditions. These conditions provide a liposomal formulation having an encapsulated nucleic acid in a ratio of at least 0.5 μg/μmole of liposomal lipid and further provides for high transfection rates with low toxicity seen, as compared to cationic lipoplexes. Applicants have demonstrated this in example 18 of the specification.

Specifically missing from the cited reference combination is the step of forming a complex of the nucleic acid and the complexing agent within the lipid stabilized water droplets, as recited in step (d) of claims 1 and 2. As explained in further detail below, the cited references do not teach or suggest that a complexing agent will react with a nucleic by way of exchange of these agents between the lipid stabilized water droplets, e.g., reverse micelles, as explained at page 27, ll. 22-27. This process is also demonstrated by the cartoon at Figure 2 of the specification. Further missing from the cited reference combination is the recited ratio of 0.5 μg nucleic acid per μmole of liposomal lipid.

The Examiner relies on Szoka for teaching a method of preparing liposomes. The method "involves preparing a solution of a phospholipid in an organic solvent, mixing with an aqueous solution of the active agent to form an emulsion and evaporation of the solvent to form unilamellar liposomes," and that "this method is valuable for the encapsulation of RNA and DNA." *Office*

Action at p. 3. The Examiner acknowledges that Szoka does not teach "the addition of a complexing agent to the emulsion containing the active agent or addition of active agent to the complexing agent containing emulsion." *Id.* at p. 3. To make up for Szoka's deficiency, the Examiner relies on either Gao or Papahadjopoulos. According to the Examiner, Gao teaches "liposomal delivery systems in which the nucleic acid is complexed with a polycation." *Office Action* at p. 3. The Examiner further contends that "one of ordinary skill in the art would be motivated to encapsulate [Gao's complex] in Skoza's liposomes because" of the high transfection ability of Gao's complex.

Neither Gao nor Paphadjopoulos describes encapsulation of a nucleic acid complexed with a polycation, as claimed. Gao describes complexing nucleic acids with *preformed, empty* cationic liposomes. Importantly, the nucleic acids are not encapsulated. Gao further does not teach that the polycation and nucleic acid are reacted in a water in oil emulsion. This step, which results in the improved transfection efficiencies of the liposomes of the present invention, is completely lacking in either Szoka or Gao. Rather, the complexation of nucleic acids with the liposomes in Gao, and optionally with a polycation, is carried out in aqueous buffer. *Id.* at col. 12, ll. 23-39. Thus, the combined teachings of these references fail to render the claimed method obvious.

Papahadjopoulos is relied upon by the Examiner for teaching a "liposomal delivery system in which nucleic aid is complexed with a polycation such as spermidine, spermine and poly amino acids." Papahadjopoulos, like Gao, also does not teach a method where the polycation and nucleic acid are reacted in a water in oil emulsion. Like Gao, Papahadjopoulos describes the preparation of lipid/DNA complex by simply combining an aqueous lipid suspension with a an aqueous buffer mixture of plasmid DNA. Specifically, to form the lipid:condensed nucleic acid complexes, a

nucleic acid is contacted with a polycation to produce the condensed nucleic acid, and then combined with an amphipathic cationic lipid. *Id.* at col. 2, ll. 25-31, see also Example 2. Thus, Papahadjopoulos preforms the condensed nucleic acid, then complexes it with a preformed liposome in aqueous solution. Like Gao, Papahadjopolous does not encapsulate a complexed bioactive agent, but rather forms an unencapsulated complex with the lipid. Furthermore, the step of reacting the complexing agent with the nucleic acid in the emulsion is missing.

The Examiner contends that Gao and Papahadjopoulos provide motivation to add a complexing agent for teaching that a nucleic acid can be complexed with a polycation, and that it would be obvious to encapsulate such complexed nucleic acids in the liposomes of Skoza. The Examiner further contends that:

> [a]lthough Szoka does not teach the formation of the emulsion first with the complexing agent and then the addition of the active agent, it would have been obvious to one of ordinary skill in the art that a complex formation would occur whether the active agent is added to the complexing agent emulsion of complexing agent is added to the active agent containing emulsion since the complexation process is between an anionic agent and a cationic agent. " *Office Action* at p. 4.

Applicants submit that this statement fails to provide any rational underpinning for the skilled artisan to perform the claimed method. Even if the skilled artisan were so motivated, none of the references provide any rational basis for carrying out the encapsulation and complexation according to the presently recited method steps, e.g., by reacting the nucleic acid and complexing agent in an emulsion with any reasonable expectation of success in achieving the claimed nucleic acid/lipid ratio.

The Examiner points to Tikchonenko as evidence that small DNA complexes can be formed under certain conditions. Tikchonenko describes unencapsulated toroid shaped DNA complexes of about 0.1 micron in size, and states that RPE liposomes generally have a diameter of about 0.3

microns, but is silent regarding the actual size of liposomes containing encapsulated complexes. *Tikchonenko* at p. 324. Tikchonenko preforms small complexes by keeping the concentration very low, *id.* at p. 323, in contrast to present method, which forms the complexes in a water in oil emulsion. Importantly, the liposomes of Tikchonenko have a concentration of about 0.02 to 0.2 micrograms per micromole of lipid. *See Shangguan et al.* Thus, the skilled artisan would not expect to form liposomes having a nucleic acid concentration of greater than 0.5 μg/μmole. The present method can be carried out with higher concentrations of nucleic acid, resulting in liposomes having a ratio of greater than 0.5 micrograms of nucleic acid per micromole of lipid. For at least these reasons, Applicants respectfully request withdrawal of this rejection.

2. Rejection over Szoka in view of Gao or Papahadjopoulos, and Tikchonenko, in further view of Kim

The Examiner also rejects claims 1-7 as being allegedly obvious over Szoka in view of either Gao or Papahadjopoulos, and Tikchonenko in further view of U.S. Patent No. 5,723,147 to Kim et al. ("Kim"). According to the Examiner, Kim discloses the preparation of liposomes in which the "lipid in an organic solvent is added with an aqueous solution of an active agent, which in turn is added, with a second aqueous solution containing a cationic amino acid lysine." *Office Action* at p. 6. Based on this, the Examiner states "[i]n essence, Kim teaches the addition of the active agent and the complexing agent by their introduction into the emulsion through two separate aqueous solutions." *Id.* Thus, the Examiner concludes that "[o]ne of ordinary skill in the art would be motivated to add the active agent such as a nucleic acid and the complexing agent through separate aqueous media to for a complex with a reasonable expectation of success since the reference of Kim shows its routine practice in the art." *Id.* Applicants respectfully traverse.

The Examiner acknowledges that lysine is not a complexing agent, but contends that Kim "is combined for the teachings of adding two aqueous solutions." *Id.* at p. 7. Applicants submit that Kim's teachings concerning lysine fail to provide the skilled artisan with any reason to use a complexing agent in this process. Furthermore, Kim's methods produces extremely large multivesicular liposomes having a diameter ± standard deviation of 19.4±6.5 microns, not liposomes having a diameter of 50-300 nm as claimed. Nothing in Kim would lead the skilled artisan to believe that a ratio of greater than 0.5 microgram per micromole lipid could be achieved with any reasonable expectation of success. For at least these reasons, Applicants respectfully request withdrawal of this rejection.

3. Rejection over Tikchonenko alone or in further view of Kim

The Examiner also rejects claims 1-7 over Tikchonenko. *Office Action* at p. 7. According to the Examiner, Tikchonenko's "method involves preparing a solution of the active and complexing agent to form an emulsion and evaporation of the solvent to form unilamellar liposomes." *Id.* The Examiner alleges that "it is unclear from Tikchonenko whether DNA and complexing agents are added separately as two aqueous solutions," but "in the absence of unexpected results, it is deemed obvious to one of ordinary skill in the art to manipulate the method of Tikchonenko to obtain the best possible results since the[] molecules are oppositely charged are expected to interact to forma a complex if added in separate solutions since the reference of Kim shows that two agent can be added separately." *Id* at p. 8. Applicants respectfully traverse.

As an initial matter, Applicants note that Tikchonenko is not "unclear" with respect to the addition of complexing agents and DNA as separate solutions or not. Tikchonenko explains that DNA was condensed with spermine prior to combination with any lipids. *Tikchonenko* at p. 323,

col. 1-2. Tikchonenko further explains that the liposomes were prepared by forming an ether solution of lipids, followed by the addition of an aqueous phase, where the aqueous phase contains "either native or condensed DNA." *Id.* at p. 322, col. 2. Thus, Tikchonenko clearly uses a preformed DNA complex, in contrast to the present claims.

Applicants note that reference must be considered in its entirety, for all that it teaches. Thus, a reference must be considered as a whole, including disclosures that away from the claimed invention. M.P.E.P. § 2142.02. Under *KSR*, "teaching away" still provides evidence of non-obviousness. *See* 127 S.Ct. at 1745. "[P]roceeding contrary to accepted wisdom in the art is evidence of nonobviousness." M.P.E.P. §2145 (citing *in re Hedges*, 783 F.2d 1083 (Fed. Cir. 1986)).

Tikchonenko concludes that all of the known problems with preparing liposomal formulations containing DNA "can be overcome by using **pre-condensed DNA preparations**." *Id.* at p. 328 (emphasis added). Thus, the skilled artisan, upon reading Tikchonenko, would understand that precondensing the DNA is essential for successful encapsulation of a nucleic acid. Kim's teachings concerning lysine, which is not even a complexing agent, fail to overcome Tikchonenko's strong teaching against the claimed invention.

For at least these reasons, Applicants respectfully request withdrawal of this rejection.

IV. Conclusion

In light of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Reconsideration and timely allowance of the pending claims is respectfully solicited. If a telephone conference would be helpful, the Examiner is invited to call

the undersigned at 617-832-1223. Please charge any additional fees required to enter this response, or credit any overpayment, to **Deposit Account No. 06-1448, TRA-016.01.**

Dated: April 23, 2008

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